

In the Specification

Please replace the paragraph spanning pages 3 through 6 with the following:

The research activities that led to the cloning of the TWIK-1 channel were carried out in the manner described below with reference to the attached sequences and drawings in which:

- SEQ ID NO: 1 represents the nucleotide sequence of the cDNA of TWIK-1 and the amino acid sequences of the coding sequence.
- SEQ ID NO: 2 represents the amino acid sequence of the TWIK-1 protein.
- Figures 1A – 1D represents the Northern blot analysis, the nucleotide sequences and the deduced amino acid sequence, as well as the hydrophobicity profile and a schematic of TWIK-1. (aA): expression of TWIK-1 mRNA in human tissues; each track contains 5 μ g of poly(A)⁺; the autoradiograph was exposed for 24 hours. (bB): SEQ ID NO: 1. CDNA sequence of TWIK-1 and the amino acid sequences of the coding sequence. The supposed transmembranal segments are circled and the P domains are underlined; o represents a potential glycosylation site and ■ represents the threonine residue in the consensus recognition site of protein kinase C. (cC): the hydrophobicity analysis and the topology of TWIK-1 deduced from it; the hydrophobicity values were calculated according to the method of Kyte and Doolittle (window size of 11 amino acids) and are presented in relation to the position of the amino acid; the shaded hydrophobic peaks correspond to the transmembranal segments. (dD): a schematic of TWIK-1, showing the configuration of the P1, P2 and M1-M4 domains.
- Figures 2A – 2B represents the sequence alignments. (aA): Highlighted portion of SEQ ID NO: 2 from Fig. 2B. alignment of the P domains of TWIK-1, TOC/YORK

and other representative K⁺ channel families; the identical and conserved residues are circled in black and in gray, respectively. (bB): SEQ ID NO: 2. alignment of TWIK-1 with potential homologues of *C. elegans*; the sequences M110.2 and F17C8.5 were deduced from the gene sequences (respective access numbers Z49968 and Z35719); the computerized splicing of the other genomic sequences of *C. elegans* (respective access numbers Z49889, P34411 and Z22180) is not sufficiently precise to allow their perfect alignment and is therefore not shown.

- Figures 3a – 3f shows the biophysical and pharmacological properties of K⁺ currents recorded by the imposed voltage technique on *Xenopus* oocytes that had received an injection of TWIK-1 cRNA; (a): the oocyte was maintained at a holding potential (HP) of -80 mV and the currents were recorded at the end of 1-s voltage jumps from -120 to +60 mV in 20 mV increments. (b): regular current-voltage relationship using the same technique as in (a). (c): potential reversal of the TWIK-1 currents (E_{rev}) as a function of the external K⁺ concentration. (d): current tracings linked to +30 mV depolarizations starting at a holding potential (HP) of -80 mV in the absence (top tracing) and in the presence (bottom tracing) of 1 mM of Ba²⁺. (e): blocking effect of 100 μ M of quinine, same protocol as in (d). (f): dose-response relationship of the blocking of the TWIK-1 currents by quinine.
- Figures 4a – 4c shows the influence of the expression of TWIK-1 on the membrane potential. (a): dose-response relationships of the cRNA; top row = equilibrium state of the outward currents measured at +30 mV; bottom row = membrane potentials associated with the resting state. (b): effect of 100 μ M of quinine on the membrane potential of an oocyte which did not receive an injection (left tracing) and that of an

oocyte that received 20 ng of TWIK-1 cRNA. (c): statistical evaluation of the depolarizing effects of 100 μ M of quinine on oocytes that did not receive injections (left bars) and on oocytes that received injections of 20 ng of TWIK-1 cRNA (right bars); control (unfilled bar), + quinine (solid bars); each bar represents the mean \pm SD of 5 oocytes.

- Figures 5a – 5c shows the properties of the single TWIK-1 channel. (a): current tracings recording in the input-output configuration to the membrane potentials indicated in the absence (m) or in the presence (·) of internal Mg^{2+} (3 mM) and in symmetry with 140 mM of K^+ . (b): mean of curves I-V ($n = 10$). (c and d): open time of distribution obtained at +80 mV (top histograms) and at -80 mV (bottom histograms) in the presence of 3 mM Mg^{2+} (c) or in the absence of Mg^{2+} (d).
- Figures 6a – 6g shows the blocking of the TWIK-1 channels by the internal pH. (a and b): blocking effect of the internal acidification on the TWIK-1 currents, induced by perfusion of CO_2 ; (a): tracings of superimposed currents induced by a depolarization phase at -30 mV starting at HP = -80 mV, control (top tracing), effect when equilibrium is reached in the presence of CO_2 (bottom tracing); (b): graph ($n = 5$) showing the almost complete blockade of the TWIK-1 currents induced by CO_2 ; (c and d): internal acidification induced by the application of DNP (1 mM). (c): same protocol as in (a), control (top tracing) and after 5 minutes of application of DNP (bottom tracing); (d): graph ($n = 4$) indicating the percentage of TWIK-1 current remaining after treatment with DNP. (e and f): imposed voltage (method: attached patch) under symmetrical conditions of K^+ concentration (140 mM) maintained at +80 mV. (e): course over time of the effect of 1 mM of DNP (marked with arrow)

on the activities of the single TWIK-1 channel. (f): graph ($n = 4$) showing the effect of DNP on the mean probability of opening NP_o calculated during 1 minute of recording starting at the equilibrium state. (g): activities measured in the “inside-out-patch” state at 80 mV at different internal pH values. Bar graph ($n = 10$) of NP_o in relation to the internal pH.

- Figures 7a – 7d shows the activation of the TWIK-1 channels by PMA, activator of protein kinase C. (a): perfusion of PMA (30 nM) for 10 minutes increases the TWIK-1 current (top tracing) induced by a depolarization phase at +30 mV starting at HP = -80 mV, control current (top tracing). (b): graph ($n = 5$) showing the activation effect of PMA on the TWIK-1 currents. (c and d): attached patch configuration under symmetrical K^+ concentration conditions maintained at +60 mV; (c): course over time of the effect of 30 nM of PMA on the single channel activities; the recordings of the channel activity were performed with a rapid scanning before and after the application of PMA; (d): bar graph ($n = 5$) showing the activation effect of PMA on NP_o .